Gold recovery from sulphide minerals: a bioprocessing approach

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Recuperación de oro de sulfuros minerales: una técnica basada en bioprocesos

Recuperació d'or de sulfurs minerals: una tècnica basada en bioprocessos

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RESUMEN

A veces la recuperación de oro de sus menas representa un reto. Esto se debe a una fina diseminación y a la incrustación del oro dentro de los sulfuros asociados. Se han ensayado numerosos métodos para solucionar este problema, incluyendo la calcinación y la oxidación además de procesos biológicos. Entre éstos últimos métodos, el uso de bacterias aumenta la biooxidación de los sulfuros y, en consecuencia, facilita su lixiviación. Por tanto, el objetico de este artículo es investigar la bio-recuperación de oro de la mena de oro de Alhura, zona situada en el Reino de Arabia Saudí. Los parámetros investigados incluyen el tamaño de la mena cargada, en mm; la cantidad de bacterias, en ml; el tiempo d retención, en días; la velocidad de rotación en rpm; el ratio de adición del nutriente bacteriano K_2SO_4 en kg/t; y el ratio de adición del nutriente bacteriano $(NH_4)_3PO_4$, en kg/t. El examen estadístico de estos parámetros indicó que los más significativos son: el tamaño de la mena de oro, la dosis de bacterias y el nutriente K_2SO_4 además del tiempo de retención. Consecuentemente, en condiciones óptimas, (10 ml de volumen de bacterias, tiempo de retención de 6 días, y 6.5 Kg/t de K_2SO_4 como alimento de las bacterias), se obtuvo un concentrado de oro conteniendo hasta 107 g/t de oro, a partir de una mena de 1,14 g/t de oro.

Palabras clave: menas de oro, sulfuros, bioprocesos, lixiviación.

SUMMARY

Sometimes gold recovery from its ores represents a challenge. This is due to fine dissemination and interlocking of the gold within the associated sulfide minerals. Many approaches were tried to solve this problem, they included roasting, oxidation in addition to bioprocessing. In the last approach, application of bacteria enhances sulfides bio-oxidation and consequently facilitates their leaching. Therefore, this paper aims at investigating gold biorecovery from Alhura area gold ore, located at Kingdom of Saudi Arabia. Investigated parameters included Feed Size, mm; Dose of bacteria, ml ; Retention time, day; Steering speed, rpm; Bacteria nutrient addition rate, K_2SO_4, kg/t; Bacteria nutrient addition rate, $(NH_4)_3PO_4$, kg/t. Statistical screening of these parameters showed that the most significant ones are: ore feed size, dose of bacteria and K_2SO_4 nutrition in addition to retention time. However at optimum conditions, (10 ml bacterial dose, 6 days retention time, and 6.5 Kg/t K_2SO_4 as bacteria nutrient) a gold concentrate containing up to 107 g/t gold from an ore containing 1.14 g/t gold was obtained.

Key words: gold ores, sulfides, bioprocessing, leaching

RESUM

De vegades la recuperació d'or de les seves menes representa un repte. Això es deu a una fina disseminació i a la incrustació de l'or de l'or en dels sulfurs associats. S'han assajat nombrosos mètodes per solucionar aquest problema, incloent la calcinació i l'oxidació a més de processos biològics. Entre aquests últims mètodes, l'ús de bacteris augmenta la biooxidació dels sulfurs i, en conseqüència, facilita la seva lixiviació. Per tant, l'objectiu d'aquest article és investigar la biorecuperació d'or de la mena d'or d'Alhura, zona situada en el Regne d'Aràbia Saudita. Els paràmetres investigats inclouen la mida de la mena carregada, en mm; el volum de bacteris, en ml; el temps de retenció, en dies; la velocitat de rotació en rpm; el ràtio d'addició del nutrient bacterià, K_2SO_4, en kg/t; i el ràtio d'addició del nutrient bacterià $(NH_4)_3PO_4$, en kg/t. L'examen estadístic d'aquests paràmetres va indicar que els més significatius són: la mida de la mena d'or carregada, el volum de bacteris i el nutrient K2SO4 a més del temps de retenció. Conseqüentment, en condicions òptimes, (10 ml de bacteris, temps de retenció de 6 dies, i 6.5 Kg/t de K_2SO_4 com a aliment dels bacteris), es va obtenir un concentrat d'or contenint fins a 107 g/t d'or, a partir d'una mena d'1,14 g/t d'or.

Paraules clau: menes d'or, sulfurs, bioprocessos, lixiviació.

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1. INTRODUCTION

Recently, the processing of low grade disseminated gold ores is in an increasing order. Therefore, this has been immensely investigated (Marais, 1990; Anon, 1993; Malhotra and Armstrong, 1993; Olson., 1994; Lorenzen, 1995; Torres and Costa, 1995; Johansson et al., 1999). The applied techniques were also documented in different review articles (Nieves and Francisco, 1995; Nilanjana, 2010). However, it was shown that the difficulty to recover gold from its ore may be attributed to one of these two major reasons:

1. Gold fine dissemination in bearing minerals such as sulfides, quartz or silicates,
2. The active carbonaceous matter content of the gold ore, which adsorb the leached gold in a cyano-complex structure and thus preventing its recovery

To overcome these reasons the diagnostic leaching technique, as a tool for gold extraction and characterization, was developed in South Africa in 1986 (Tumilty and Schimidt, 1986), and is now widely used to provide a good indication of gold deportation in its various occurrence phases (Henley, 1989). To improve the ability of the technique to deal with refractory gold ores different modifications were tried (Whitlock, 1997; Amankwah et al., 2005). These include:

- Oxidative roasting as a pre-oxidation step before cyanidation to remove harmful active carbonaceous constituents by oxidation or volatilization.
- Biooxidation pretreatment especially for sulfidic gold ores to enhance leaching and to decompose the sulfide matrix, thus exposing the encapsulated precious metals to leaching solution.

Bio-leaching or bacterial leaching is fundamentally a process which is the result of a variety of bacteria oxidizing sulfide host minerals to liberate metal values. In the case of pyrite, the following equations describe the typical reactions that occur in a bacterially-catalyzed oxidation system (Ubaldini et al., 2000)).

$$2FeS_2 + 7O_2 + 2H_2O \longrightarrow 2FeSO_4 + 2H_2SO_4$$
$$4FeSO_4 + O_2 + 2H_2SO_4 \longrightarrow 2Fe_2(SO_4)_3 + 2H_2O$$
$$FeS_2 + Fe_2(SO_4)_3 \longrightarrow 3FeSO_4 + S^\circ$$
$$2S^\circ + 2H_2O + 3O_2 \longrightarrow 2H_2SO_4$$
$$Fe_2(SO_4)_3 + 6H_2O \longrightarrow 2Fe(OH)_2 + 3H_2SO_4$$

Bioleaching can take place in the form of heap leaching or stirred tank leaching. Although, the bioleaching in stirred tanks at industrial scale is limited to sulfidic concentrates, for recovery of gold and base metals, it has two main advantages:1) the economic potential for its application into industrial scale, 2) microbial growth selectivity in the bioreactors (Dominique, 2007; Ciftci and Akcil, 2010).

Many Saudi gold ores are often found as very finely disseminated particles in the sulfide matrix which hampers their extraction by cyanidation. To improve the direct leaching of such difficult-to-be leached sulfides, high doses of cyanide are used with no trials made to test more advanced leaching techniques as bioleaching. Therefore, in this paper, the bioleaching in agitated tank of Al Masane gold deposits was investigated. The significant parameters affecting the process were optimized. The stepwise optimization was used staring with Plackett–Burman screening design to address the most significant factors and followed by surface response design, Box-Behnken design to determine the optimum levels of significant parameters.

2. MATERIALS AND METHODS

2.1. The ore samples

An ore sample, for this study, was selectively chosen from Al Masane Alkobra gold deposits which are located in southwestern Saudi Arabia. The deposit area is located approximately 640 km southeast of Jeddah at latitude 18°08›N and longitude 43°51›E. The samples were selected and picked so as to be of major sulfide contents.

2.2. Stirred tank bio-leaching process

Stirred tank bio-leaching process applied in this work was carried out in a typical procedure described elsewhere (Attia and El-Zeky, 1989). Where, the key components of the process involve concentration of a ground ore, usually by flotation, followed by oxidation of the gold-bearing sulfides to the required extent in a multi-stage process. Oxidized solids discharged from the last reactor are separated from the bio-leachate, washed and cyanide leached for gold extraction in a conventional carbon in pulp (CIP) or other cyanidation circuit. The bio-leachate, usually containing primarily soluble iron and arsenic in addition to acid, is treated by controlled lime neutralization to produce stable precipitates for disposal.

2.3. The Used Bacterium

Thiobacillus ferrooxidans was used in this work for oxidation of sulfide ores before cyanidation. The bacteria were locally prepared at the same procedure explained by Natal'ya and coworkers (Natal'ya et al., 2010). *Thiobacillus ferrooxidans* has a cell size in the range of 0.5 to 0.6 μm long (Lewis, 1990). Its energy sources are ferrous iron and reduced sulfur. It will oxidize virtually all known sulfide minerals, including pyrite, arsenopyrite, copper sulfides etc. It thrives in the pH range 1.0 to 6.0, the optimum pH for maximum growth rate being between 2.0 to 2.5. Similarly, it survives in the temperature range 2 °C to 40 °C, but its maximum efficiency was found within the range of 28–35 °C (Natal'ya et al., 2010). This bacterium is robust, thriving in H_2SO_4 environments at pH less than 2.5. None of these bacteria has been found to cause plant or animal diseases (Ciftci and Akcil, 2010).

2.4. Statistical Design Methodology

Screening and central composite statistical experimental designs are used to determine the effect of various factors on the gold extraction. The statistical software package Design-Expert 6.1, Stat-Ease, Inc., Minneapolis, USA was used for regression analysis of experimental data and to plot response surface. ANOVA was used to estimate the statistical parameters.

2.3.1. Screening Design (Plackett-Burman Design)

The PlackettBurman design (PB), (Plackett and Burman, 1946) was used to screen out the most statistically significant process factors. PB design is based on the first order model:

$$Y=\beta_o + \Sigma\, \beta_i x_i$$

Where Y is the response (gold g/t, gold recovery%, yield %), b_o is the model intercept and b_i are the coefficients, and x_i are the levels of the independent variables. The variables examined, in the screening stage and the design matrix, were shown in Table 1.

2.3.2. Optimization using Box-Behnken design

A Box-Behnken design (Box and Behnken, 1960) was used to further investigate the three most significant factors determined from the screening design. The design-matrix of different runs, 15 experiments, is shown in Table

2. According to this design, the optimal conditions were estimated using a second order polynomial function by which a correlation between studied factors and response was generated. The general form of this equation is:

$$Y=\beta_{0+}\beta_1X_1 + \mathbf{b}_2X_{2+}\mathbf{b}_3X_3 + \mathbf{b}_{12}X_1X_2 + \mathbf{b}_{13}X_1X_3 + \mathbf{b}_{23}X_2X_3 + \mathbf{b}_{11}X_1^2 + \mathbf{b}_{22}X_2^2 + \mathbf{b}_{33}X_3^2$$

Where Y is the predicted response (the gold content (g/t), gold recovery % and yield %), X_1, X_2 and X_3 are studied variables; $\mathbf{b}_i$ are equation constants and coefficients.
Software package, Design-Expert 6.0.5, Stat-Ease, Inc., Minneapolis, USA, was used for regression analysis of experimental data and to plot response surface. Analysis of variance (ANOVA) was used to estimate the statistical parameters. The extent of fitting the experimental results to the polynomial model equation was expressed by the determination coefficient, R^2. F-test was used to estimate the significance of all terms in the polynomial equation within 95% confidence interval.

3. RESULTS AND DISCUSSION

3.1. Chemical Analysis

The chemical analysis of selected samples from Al-Houra locality is given in Table 3. It shows that the ore contains a high amount of silver. In addition mineralogical investigation of the ore shows that it has a high chalcopyrite and arsenopyrite portions.

Table 1. *Experimental Plackett-Burman (PB) design for 11 variables*

Run No.	Variable Levels										
	1	2	3	4	5	6	7	8	9	10	11
1	-	-	+	+	+	-	+	+	-	+	-
2	+	-	+	-	-	-	+	+	+	-	+
3	-	-	-	-	-	-	-	-	-	-	-
4	+	+	+	-	+	+	-	+	-	-	-
5	+	-	+	+	-	+	-	-	-	+	+
6	+	-	-	-	+	+	+	-	+	+	-
7	+	+	-	+	-	-	-	+	+	+	-
8	+	+	-	+	+	-	+	-	-	-	+
9	-	+	+	+	-	+	+	-	+	-	-
10	-	-	-	+	+	+	-	+	+	-	+
11	-	+	-	-	-	+	+	+	-	+	+
12	-	+	+	-	+	-	-	-	+	+	+

Variables	Low Level (-)	High Level (+)
1)Ore feed size,	-0.025	-0.075
2)Dose of bacteria, ml	1	10
3) dummy ♣		
4) dummy		
5)Retention time, day	1	10
6)Solid / Liquid ratio	1	10
7)Steering speed, rpm	500	2500
8) dummy		
9)Bacteria nutrient addition rate, K_2SO_4, kg/t	1	12
10)Bacteria nutrient addition rate, $(NH_4)_3PO_4$, kg/t	0.2	2
11) dummy		

♣dummy variables are used to calculate the variance of the experimental runs

3.2. Process Statistical Analysis

3.2.1. Optimization of the bioleaching process

3.2.1.1. Screening statistical design

PB design offers good and fast screening procedure and mathematically computes the significance of large number of factors in small number of experiments. In this study, the gold in concentrate and its recovery, Table 4, were used as responses in calculating the statistical effect of variables. The effect of variables is shown in Table 5.

Table 2. *Box-Behnken (3 levels and 3 variables)*

Run No.	Bacteria dose, ml	Retention time, day	Bacteria nutrient addition rate, K_2SO_4, kg/t
1	**-1**	**-1**	**0**
2	**-1**	**+1**	**0**
3	**+1**	**-1**	**0**
4	**+1**	**+1**	**0**
5	**-1**	**0**	**-1**
6	**-1**	**0**	**+1**
7	**+1**	**0**	**-1**
8	**+1**	**0**	**+1**
9	**0**	**-1**	**-1**
10	**0**	**-1**	**+1**
11	**0**	**+1**	**-1**
12	**0**	**+1**	**+1**
13	**0**	**0**	**0**
14	**0**	**0**	**0**
15	**0**	**0**	**0**
Levels			
Variables	**-1**	**0**	**+1**
Dose of bacteria, ml	**1**	**5.5**	**10**
Retention time, day	**1**	**5.5**	**10**
Bacteria nutrient addition rate, K_2SO_4, kg/t	**1**	**6.5**	**12**

Table 3*. Chemical Analysis of selected samples from Al-Houra Locality*

Constituent (unit)	Zn (%)	Cu (%)	Au (g/t)	Ag (g/t)
Value	3.98	0.99	1.14	35.3

Table 4. *PB results for gold assay and its recovery corresponding to the different carried tests*

Test	1	2	3	4	5	6	7	8	9	10	11	12
Gold, g/t	83	86	91	76	114	91	105	60	99	81	94	103
Gold Rec., %	45.4	48.6	52.8	34.9	79.9	49	55	24.9	61.2	38.9	48.2	66.2

Table 4 shows that run #5 gives the best gold g/t as well as gold recovery %. By analyzing the PB designs for both responses, i.e., gold and its recovery show that there is a coincidence between the trends of both designs in terms of the significant factors affecting the extraction process. Therefore, the gold recovery ANOVA table, Table 5, was chosen as an example of such statistical analysis. The sig-

nificance of the main affecting factors was estimated by analysis of variance using F-tests, Table 5. The model F-value of 362.20 implies that the model is significant. There is only a 0.28% chance that a "Model F-Value" this large could occur due to noise. In deciding the significant parameters of the process, the values of "Prob > F" were considered where values less than 0.0500 indicate significance. In this case, B (Dose of bacteria, ml , C (Retention time, day), F (Bacteria nutrient addition rate, K_2SO_4, kg/t) , and G (Bacteria nutrient addition rate, $(NH_4)_3PO_4$, kg/t) are the most significant parameters. In addition, the standard deviations are 0.87, 0.84 and correlation coefficients (R-Squared) are 0.9997, 0.9994 for gold and its recovery, respectively. The obtained results is in good agreement with work done by Chapman (Chapman et al., 1993). Nevertheless, the both nutrients are significant, the bacteria nutrient addition rate, K_2SO_4, kg/t was used in optimization of the extraction process due to to its higher effect on the gold recovery, Table 5.

Table 5 *Gold recovery, % Analysis of variance (ANOVA) [Partial sum of squares]*

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F	
Model	2321.70	9	257.97	362.2	0.0028	significant
A	11.42	1	11.42	16.04	0.0571	
B	319.73	1	319.73	448.91	0.0022	
C	547.76	1	547.76	769.08	0.0013	
E	6.29	1	6.29	8.83	0.0971	
F	77.71	1	77.71	109.11	0.0090	
G	34.79	1	34.79	48.84	0.0199	
H	212.65	1	212.65	298.57	0.0033	
K	978.70	1	978.70	1374.15	0.0007	
L	132.65	1	132.65	186.25	0.0053	
Residual	1.42	2	0.71			
Cor Total	2323.12	11				

Where studied parameters are coded as follows;
A: Feed Size, mm, B : Dose of bacteria, ml , C: Retention time, day, E: Steering speed, rpm, F : Bacteria nutrient addition rate, K_2SO_4, kg/t , G : Bacteria nutrient addition rate, $(NH_4)_3PO_4$, kg/t and H, K, L : Dummy variables

3.2.1.2. Box-Behnken optimization

Box-Behnken Design (BBD) results of gold concentration are given in Table 6, and Figures 1, 2 and 3 in terms of gold g/t, gold recovery % and yield % in the concentrate fraction respectively within 95% confidence interval. Statistical testing of the model has been done by the Fisher's statistical test for ANOVA—the analysis of variance. The standard deviation and R-Squared for each response are: 1.56, 0.9183; 2.47, 0.9661 ; 1.76, 0.9794, repectively. The R-squared suggested that there is an excellent agreement between the experimental and predicted values obtained from the model.

Table 6 shows a maximum gold content (108 g/t) was observed when the dose of bacteria (ml), Retention time(day) and Bacteria nutrient addition rate, K_2SO_4 (kg/t) were at +1, 0 and +1 levels, respectively (run #8), however, the gold recovery % is not the maximum at this run. It is interesting to notice that the run #4 represents the best conditions in terms of all measured responses. Although the run # 8 is higher in the gold content by one unit than run #4, this difference is considered statistically insignificant.

Second order regression equations (1-3) show the dependence of various responses on the studied variables. It is interesting to notice that the determined responses not only depend on the main effect of the studied variables but also they depend on the interaction between the studied variables.

$$Y1 = 99.6 - 0.96*A + 1.21* B - 0.09* C + 0.124 *A2 - 0.075* B2 - 0.024* C2 - 0.025* A*B + 0.031*A*C + 0.051* B*C \quad (1)$$

$$Y2 = 38.34 - 2.05*A + 2.069*B + 5.88*C + 0.28*A2 - 0.082* B2 - 0.35*C2 - 0.02*A*B - 0.023*A*C -0.08*B*C \quad (2)$$

$$Y3 = + 43.56 - 1.7*A + 2.02*B + 5.98* C + 0.263*A2 - 0.07*B2 - 0.382* C2 - 0.076*A* B - 0.058*B*C \quad (3)$$

Where is the Yi is the predicted response (Gold, g/t; Gold recovery, %; Yield, %), A the coded value of Bacterial dose, ml; B the coded value of Retention time, day and C the coded value of Bacteria nutrient addition rate, K_2SO_4, kg/t.

Table 6 *Results of Box-Behnken design experimental runs*

Run No.	A	B	C	Gold, g/t	Gold recovery, %	Yield, %
1	1	1	6.5	100	59.55	64.65
2	10	1	6.5	105	67.64	75.6
3	1	10	6.5	104	68.02	74.56
4	10	10	6.5	107	74.48	79.36
5	1	5.5	1	103.1	51.88	57.36
6	10	5.5	1	106	59.55	62.86
7	1	5.5	12	102	58.36	63.36
8	10	5.5	12	108	63.77	69.9
9	5.5	1	1	98	42.90	49.9
10	5.5	10	1	103	48.85	54.6
11	5.5	1	12	96	57.28	61.6
12	5.5	10	12	106	55.27	60.58
13	5.5	5.5	6.5	102	62.50	68.5
14	5.5	5.5	6.5	104	63.77	69.9
15	5.5	5.5	6.5	103	64	70.4

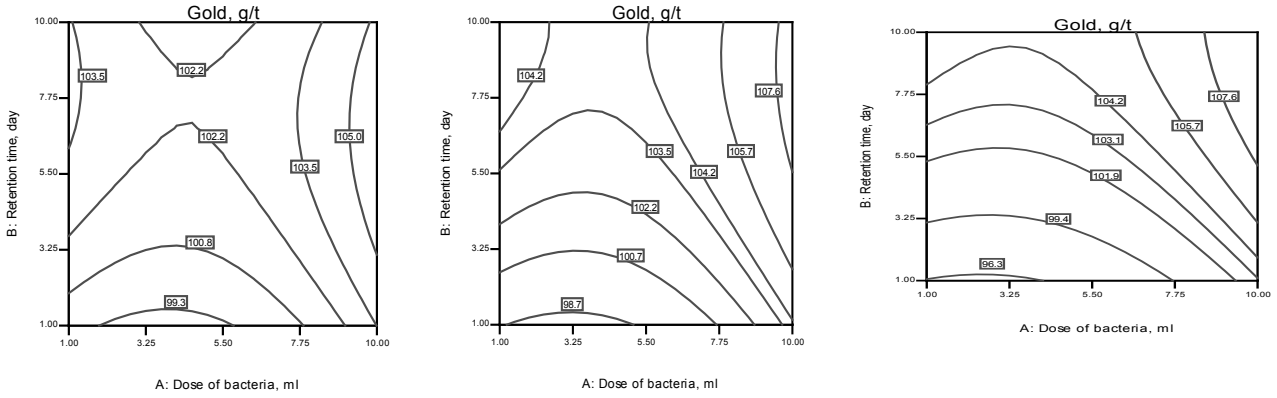
A: Bacteria dose, ml, B: Retention time, day, C: Bacteria nutrient addition rate, K_2SO_4, kg/t

3.2.1.3. Interaction of studied variables

Figure 4 represents the interaction plots for the effects of dose of bacteria and retention time on the gold content, gold recovery and yield at low and high levels of bacteria nutrition rate, K_2SO_4, i.e., 1 and 12 kg/t, respectively. It can be seen from Fig.4 that the studied responses are greatly and significantly affected by their interaction. It is also noticed that the effect of the factors interaction on the gold recovery and yield. However, the dose of bacteria is significantly affecting all the variables after the mid-point level. This agrees with what was noticed by Ubaldini and his coworkers (Ubaldini et al., 2000).

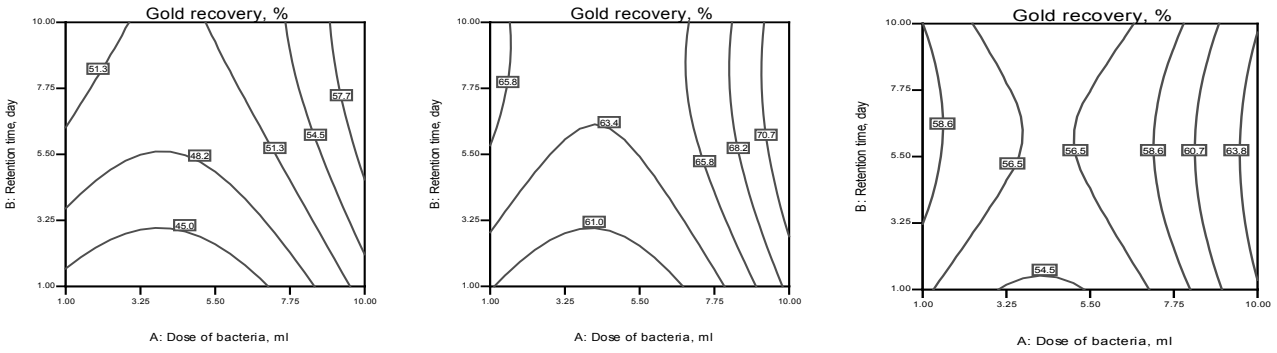
4. CONCLUSIONS

The bioleaching was tested as a pretreatment process to cyanidation process. The optimization of the process was conducted using Plackett-Burman screening design followed by surface response optimization using Box-



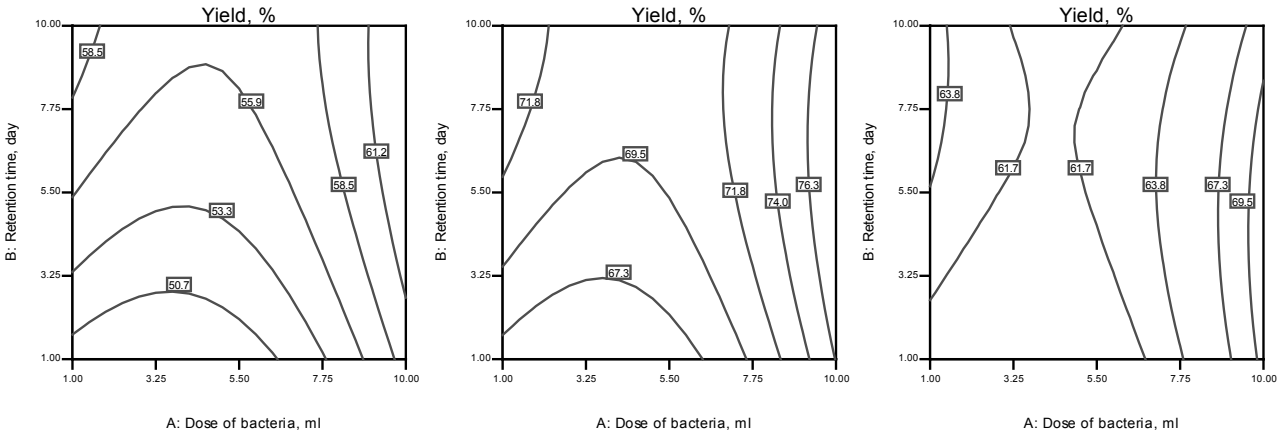
a) *b)* *c)*

Figure 1 Gold content in terms of dose of bacteria and retention time as function of Bacterial nutrition rate, K_2SO_4, kg/ t : a) 1 b) 6.5 c)12



a) *b)* *c)*

Figure 2 Gold recovery in terms of dose of bacteria and retention time as function of Bacterial nutrition rate, K_2SO_4, kg/ t : a) 1 b) 6.5 c)12



a) *b)* *c)*

Figure 3 Yield % in terms of dose of bacteria and retention time as function of Bacterial nutrition rate, K_2SO_4, kg/ t : a) 1 b) 6.5 c)12

Behnken design. The screening design was used to identify the significant factors among the various parameters included in the process. The important and significant factors are further optimized using Box-Behnken design. The experimental results clearly showed that the gold recovery is dependent mainly on dose of bacteria, retention time and nutrition K_2SO_4.

Optimum conditions at which the best gold content as well as gold recovery are 10 ml bacterial dose, 6 days retention time, and 6.5 Kg/t K_2SO_4 nutrition, respectively. At these conditions the gold and its recovery were 107 % and 74% respectively. The quadratic model could be used to study the dependence of studied responses on different variables.

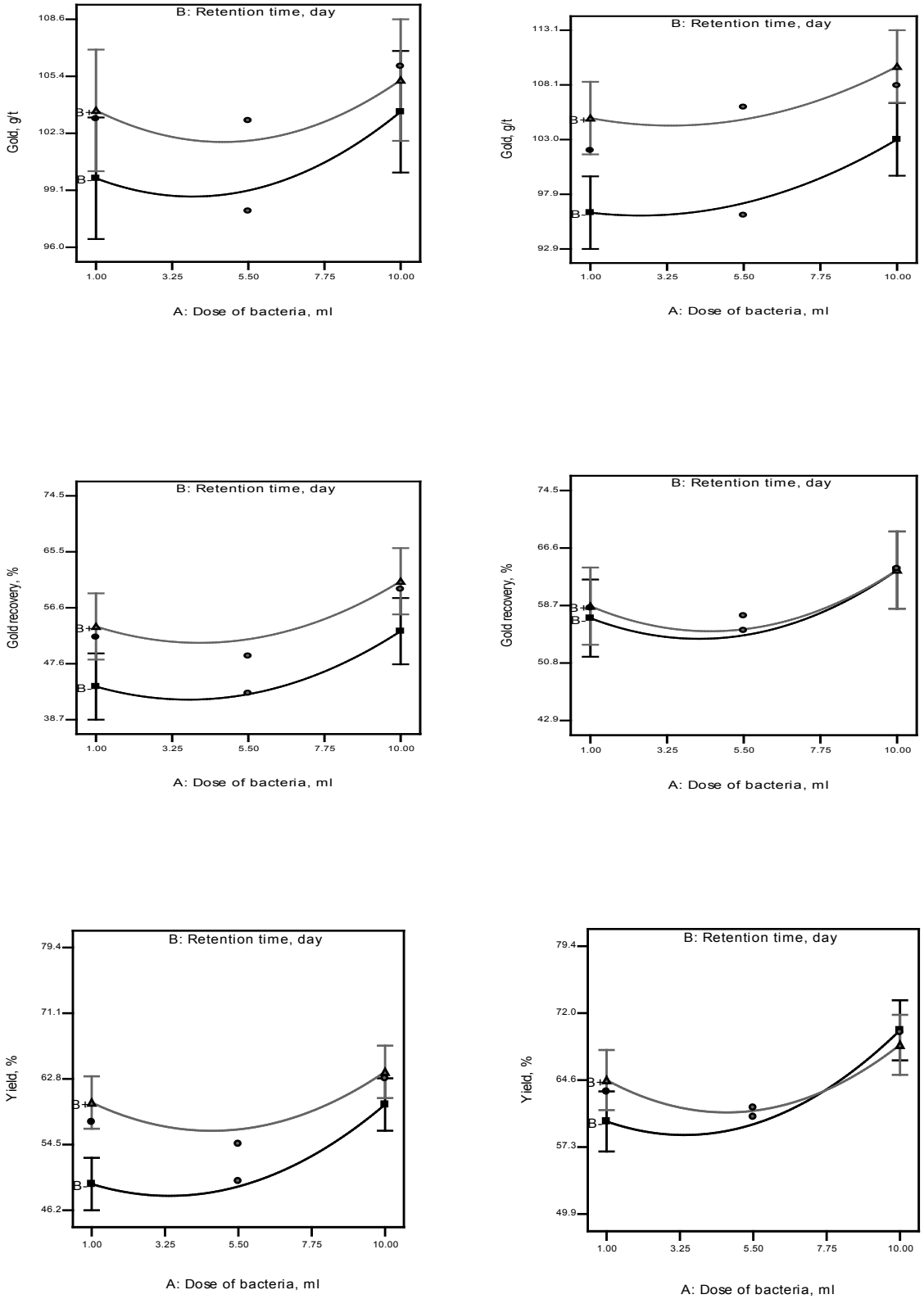


Figure 4 Interaction graph of Gold content, gold recovery and yield at low and high level of bacteria nutrition K_2SO_4

5. BIBLIOGRAPHY

1. Amankwah, R. K.; Yen, W. T.; Ramsay, J. A.: "A two-stage bacterial pretreatment process for double refractory gold ores", Minerals Engineering, **18** (1), 103-108 (2005).
2. Anon.: "Refractory gold", Engineering and Mining Journal, 20-24 (1993).
3. Attia, Y.A.; El-Zeky, M.; "Bioleaching of gold pyrite tailings with adapted bacteria", Hydrometallurgy, **22** (3), 291-300 (1989).
4. Box, G.; Behnken, D.: "Some new three level designs for the study of quantitative variables", Technometrics, **2**, 455–475 (1960).
5. Chapman, J.T.; Marchant, P.B.; Lawrence, R.W.; Knopp, R.: "Bio-oxidation of a refractory gold bearing high arsenic sulphide concentrate: A pilot study", FEMS Microbiology Reviews, **11** (1-3), 243-252 (1993).
6. Ciftci, H.; Akcil, A.: "Effect of biooxidation conditions on cyanide consumption and gold recovery from a refractory gold concentrate", Hydrometallurgy, **104** (2), 142-149 (2010).
7. Henri, D.; Morin, R.: "Bioleaching Of Sulfide Minerals In Continuous Stirred Tanks", in Donati, E.R.and Sand, W. (eds.), Microbial Processing of Metal Sulfides, 133–150, Springer, New York (2007).
8. Henley, K.J.: "A Combined Mineralogical / Metallurgical Approach to Determining the Nature and Location of Gold in Ores and Mill Products", Minerals Engineering, **2** (4), 459-470 (1989).
9. Johansson, C.; Shrader, V.; Sussa, J.; Adutwum, K.; Kohr, W.: "Biohydrometallurgy and the Environment towards the Mining of the 21st century", in, Amils, R. and Ballester, A. (eds.), 569, Elsevier, Amsterdam. (1999)
10. Lewis, P. J.: "Treatment of oxidised and primary copper/gold ores at Red Dome, Queensland, Australia", in "RANDOL SQUAW VALLEY '90". Proceedings. Golden, Randol International Ltd., 59-65. (1990)
11. Lorenzen, L: "Some guidelines to the design of a diagnostic leaching experiment", Minerals Engineering, **8** (3), 247-256 (1995).
12. Malhotra, D. and Armstrong, S.: "Characterization of Refractory Gold Ores through Diagnostic Leach Procedures", Trans. SME, 1993 SME Annual Meeting (1993).
13. Marais H. J.: "Innovation in metallurgical plant", South African Institute of Mining and Metallurgy, Johannesburg, 125 (1990).
14. Natal'ya, V.; Fomchenko, M.; Muravyov, I.; Kondrat'eva, T.F.: "Two-stage bacterial–chemical oxidation of refractory gold-bearing sulfidic concentrates", Hydrometallurgy, **101** (1-2), 28-34 (2010).
15. Nieves, I. and Francisco, C.: "Refractory gold-bearing ores: a review of treatment methods and recent advances in biotechnological techniques", Hydrometallurgy, **34** (3), 383-395 (1994).
16. Nilanjana, D.: "Recovery of precious metals through biosorption - A review", Hydrometallurgy, **103** (1-4), 180-189 (2010).
17. Olson, G. J.: "Microbial oxidation of gold ores and gold bioleaching", FEMS Microbiology Letters, **119** (1-2), 1-6 **(**1994).
18. Plackett, R. L. and Burman, J. P.: "The Design of Optimum Multifactorial Experiments", Biometrika, **33** (4), 305-325 (1946).
19. Torres, V. M.; Costa, R.S.: "Characterization of gold ores and CIP tailings using a diagnostic leaching technique". In: "XIX INTERNATIONAL MINERAL PROCESSING CONGRESS" Proceedings. Littleton, Society for Mining, Metallurgy and Exploration Inc., **1**, 15-18 (1995).
20. Tumilty, J.A.; Schimidt, C.G. : "Deportment of gold in the Witwatersrand System". in: "GOLD 100 International Conference On Gold" Proceedings. Johannesburg, SAIMM, 541-553 (1986).
21. Ubaldini, S.; Vegliò, F.; Beolchini, F.; Toro, L.; Abbruzzese, C.: "Gold recovery from a refractory pyrrhotite ore by biooxidation", International Journal of Mineral Processing, **60** (3-4), 247-262 (2000).
22. Whitlock, J. L.: "Biomining: Theory, Microbes and Industrial Processes", D. Rawlings, D. (ed.), 17, Springer, New York (1997).